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BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Paper No. 03222004

Application Number: 09/935,168
Filing Date: August 21, 2001
Appellant(s): WEST ET AL.

Rivka D. Monheit
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed 2/3/04.

(1) Real Party in Interest

A statement identifying the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

A statement identifying the related appeals and interferences which will directly affect or be directly affected by or have a bearing on the decision in the pending appeal is contained in the brief.

(3) Status of Claims

The statement of the status of the claims contained in the brief is incorrect. A correct statement of the status of the claims is as follows:

Claims 3-5 are withdrawn from consideration as not directed to TGFβ under examination.

Claims 1-2, and 6-9 are on appeal.

(4) Status of Amendments After Final

The status of amendments after final rejection contained in the brief is correct.

(5) Summary of Invention

The summary of invention contained in the brief is correct.

(6) Issues

The appellant's statement of the issues in the brief is partially correct. The change is as follow:

The rejection of claim 2 under 35 U.S.C. § 103(a) as obvious over WO 94/23740 in view of Dinbers (J Biol Chem 274(47): 29822-29829, 1996, is hereby withdrawn in view of the cells are not attached to scaffold in either reference.

The issue presented on appeal are:

(1) Whether claims 1 and 6-9 were properly rejected under 35 U.S.C. § 103(a) as obvious over WO 94/23740 in view of Dinbers (J Biol Chem 274(47): 29822-29829, 1996.

(2) Whether claims 1-2 and 6-9 were properly rejected under 35 U.S.C. § 103(a) as obvious over WO96/27657 in view of Dinbers (J Biol Chem 274(47): 29822-29829, 1996.

(7) Grouping of Claims

Appellant's brief includes a statement that the claims do not stand or fall together. The claims can be group as follows: (1) claim 1; (2) claim 2; (3) claims 3 and 5; (4) claim 4; (5) claim 6; and (6) claims 7-9.

(8) Claims Appealed

A substantially correct copy of appealed claims 1-9 appears on page 17 of the Appendix to the appellant's brief. The minor errors are as follows: claims 3-5 are not on appeal because said claims are withdrawn from consideration as not directed to TGFβ under examination.

(9) Prior Art of Record

WO 94/23740	Bentz et al	10-1994
WO 96/27657	Cima et al	9-1996

Dingers et al, J Biol Chem 271(47): 29822-29, 1996

(10) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

The method for making a tissue engineering scaffold wherein the matrix enhancing molecule is TGFβ is under examination.

Claim 1 merely requires when the matrix enhancing molecule is TGFβ, the TGFβ is coupled to a matrix by a polymer tether having a molecular weight between 2000 and 6000 and is in a density between 1 and 100 ng TGF-β/ml and does not require the growth factor TGFβ be covalently coupled to a polymeric scaffold or cell bound to the scaffold.

Claim Rejections - 35 USC § 103

1. Claims 1, and 6-9 are rejected under 35 U.S.C. 103(a) as being unpatentable over WO 94/23740 (Oct 1994, PTO 1449) in view of Dinbergs *et al* (J Biol Chem 271(47): 29822-29, 1996; PTO 892).

The WO 94/23740 publication teaches a method for making a tissue engineering scaffold such as bone formation (See page 7, line 9-13, in particular) comprising coupling various matrix-enhancing molecules such as TGFβ or TGFβ2 to a polymer matrix such as polyethylene glycol having a molecule weight such as 5000 (M-S-PEG 5000) which is between 2000 and 6000 (See page 12, lines 12-14, PEG-TGF-β conjugates, rhTGF- TGF-β2 (PEG5000) bridging page 13, in particular). The WO 94/23740 publication further teaches that the reference matrix-enhancing molecule TGF-β is covalently coupled to tether or linking group such as hydroxysuccinimide to the scaffold such as the hydrophilic polymer such as polyethylene glycol (PEG5000) (See page 11, lines 10-28, in particular). The reference method of making a tissue engineering scaffold comprises coupling TGFβ to a polymer which is useful for stimulation of bone formation at a lower dose (See abstract, page Summery of invention, in particular). The WO 94/2370 publication teaches that the method results affect variable (See abstract, page 2, lines 11-15, in particular).

The claimed invention in claim 1 differs from the teachings of the reference only that the method for making a tissue engineering scaffold without increasing cellular proliferation and the TGF-β is in a density between 1 and 100 ng/ml.

The claimed invention in claim 7 differs from the teachings of the reference only that the method wherein the scaffold is a hydrogel.

The claimed invention in claim 8 differs from the teachings of the reference only that the method wherein the hydrogel is a formed of a polymer selected from the group consisting of alginate, collagen, and hyaluronic acid.

The claimed invention in claim 9 differs from the teachings of the reference only that the method wherein the matrix enhancing molecules are TGF-β is in a concentration between about 4 x 10^{-6} to 4 x 10^{-3} nmol/ml.

Dinbergs *et al* teach a method for making a tissue engineering scaffold or formation of extracellular matrix by cells such as endothelial cells or smooth muscle cells seeded to the scaffold such as plastic of a 12-well tissue cultured plate. The reference method comprises coupling various matrix-enhancing molecule such as bFGF or TGFβ to a polymer such as alginate Heparin Sepharose microphere (See page 29823, Alginate/Heparin-Sepharose Microsphere Preparation and Growth Factor Incorporation, page 29823, column 2, bridging page 29824 column 1, in particular). The coupling of TGFβ to the polymer is at a concentration of 3 ng/sphere and 5-10 Evac TGFβ1 microspheres are placed in 1 ml, which is equivalent to 15-30

ng/ml that is within the claimed limit of a density between 1 and 100 ng TGFβ/ml (See page 29823, fourth paragraph, in particular). The reference concentration of TGFβ coupled to the polymer tether is *without* increasing cellular proliferation or cell number (See page 29825, Figure 3A (Endothelial cell) and 3B (smooth muscle cell), comparing filled circle to the open circle which is the control, in particular). Dinbergs *et al* further teach that TGFβ has been incorporated into scaffold or various biodegradable polymer matrix such as collagen, hydrogels such as alginate, hydron (hyaluronic acid) and polyethylene glycol polymers (See page 29827, column 2, first full paragraph, in particular). Dinbergs *et al* teach TGFβ is useful for eliciting extracellular matrix formation without increasing cellular proliferation for up to five days when coupling to various polymer such as alginate hydrogel for a sustained release (See page 29825, Fig 3A, in particular).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute the TGF-beta-2 and the polyethylene glycol as taught by the WO 94/23740 publication for the TGFβ at a concentration between 15-30 ng/ml and the hydrogel such as alginate, respectively, as taught by Dinbergs *et al* for a method of for making a tissue engineering scaffold without increasing cellular proliferation and the TGF-β is in a density between 1 and 100 ng/ml as taught by the WO 94/23740 publication and Dinbergs *et al.* From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention.

One having ordinary skill in the art would have been motivated to do this because the WO 94/23740 publication teaches that coupling TGFβ to a polymer is useful for stimulation of bone formation and the method results affect by variable (See abstract, page 8, in particular). Dinbergs *et al* teach that TGFβ coupling to polymer at a concentration of 15-30 ng/ml is without increasing cellular proliferation or cell number (See page 29825, Figures3A (Endothelial cell) and 3B (smooth muscle cell), comparing filled circle to the open circle which is the control, in particular). Claim 9 is included in this rejection because 4×10^{-6} to 4×10^{-3} nmol/ml is equivalent to between 5 and 100 ng/ml and Dinbergs *et al* teach TGFβ at 15-30 ng/ml is effective for inducing formation of extracellular matrix by endothelial cell or smooth muscle cell without increasing cell proliferation.

2. Claims 1-2, and 6-9 are rejected under 35 U.S.C. 103(a) as being unpatentable over WO 96/27657 (Sept 1996; PTO 1449) in view of Dinbergs *et al* (J Biol Chem 271(47): 29822-29, 1996; PTO 892).

The WO 96/27657 publication teaches a method for making a tissue engineering scaffold comprising coupling various matrix-enhancing molecules such as TGFβ (see page 10, claim 25 of WO 96/27657 publication, in particular) flexibly linked or tethered (See page 6, line 11, page 12, Attachment methods, in particular) to substrate such as polymer matrix such as bottles, dishes, fibers, shaped polymers, particles, microparticles (See page 9, lines 25-26, in particular), tissue regeneration devices such as collagen, or polyethylene oxide, alginate via carbodiimides as a cross-linker (See page 17, lines 1-12, in particular). The reference polymer tether has a molecular weight 3000-12,000 (See page 7, line 15, in particular). The reference method further attaches cells to the reference scaffold (See page 16, line 7, in particular) for constructing tissue regeneration such as production of extracellular matrix proteins such as collagen (See page 17, line 1-4, in particular). The WO 96/27657 publication teaches the growth factor is localized to desired target cell population and significantly less growth factor is needed to exert a biologic response (See abstract, in particular).

The claimed invention in claim 1 differs from the teachings of the reference only that the method for making a tissue engineering scaffold without increasing cellular proliferation and the TGF-β is in a density between 1 and 100 ng/ml.

The claimed invention in claim 9 differs from the teachings of the reference only that the method wherein the matrix enhancing molecules are TGF-β is in a concentration between about 4 x 10^{-6} to 4 x 10^{-3} nmol/ml.

Dinbergs *et al* teach a method for making a tissue engineering scaffold or formation of extracellular matrix by cells such as endothelial cells or smooth muscle cells seeded to the scaffold such as plastic of a 12-well tissue cultured plate. The reference method comprises coupling various matrix-enhancing molecule such as bFGF or TGFβ to a polymer such as alginate Heparin Sepharose microphere (See page 29823, Alginate/Heparin-Sepharose Microsphere Preparation and Growth Factor Incorporation, page 29823, column 2, bridging page 29824 column 1, in particular). The coupling of TGFβ to the polymer is at a concentration of 3 ng/sphere and 5-10 Evac TGFβ1 microspheres are placed in 1 ml, which is equivalent to 15-30 ng/ml that is within the claimed limit of a density between 1 and 100 ng TGFβ/ml (See page 29823, fourth paragraph, in particular). The reference concentration of TGFβ coupled to the

polymer tether is *without* increasing cellular proliferation or cell number (See page 29825, Figure 3A (Endothelial cell) and 3B (smooth muscle cell), comparing filled circle to the open circle which is the control, in particular). Dinbergs *et al* further teach that TGFβ has been incorporated into scaffold or various biodegradable polymer matrix such as collagen, hydrogels such as alginate, hydron (hyaluronic acid) and polyethylene glycol polymers (See page 29827, column 2, first full paragraph, in particular). Dinbergs *et al* teach that TGFβ is useful for eliciting extracellular matrix formation without increasing cellular proliferation for up to five days when coupling to various polymer such as alginate hydrogel for a sustained release (See page 29825, Fig 3A, in particular).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to use TGFβ at a concentration of 15-30 ng/ml as taught by Dinbergs *et al* for a method of making a tissue engineering scaffold for inducing formation of extracellular matrix by cells bound to the scaffold without increasing cellular proliferation wherein the TGF-β is at a density between 1 and 100 ng/ml as taught by the WO 96/27657 publication and Dinbergs *et al.* From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention.

One having ordinary skill in the art would have been motivated to do this because the WO 96/27657 publication teaches that when growth factor is localized to desired target cell, significantly less growth factor is needed to exert a biologic response (See abstract, in particular). Dinbergs *et al* teach that TGFβ coupling to polymer at a concentration of 15-30 ng/ml is without increasing cellular proliferation or cell number (See page 29825, Figures3A (Endothelial cell) and 3B (smooth muscle cell), comparing filled circle to the open circle which is the control, in particular). Claim 9 is included in this rejection because between 4×10^{-6} to 4×10^{-3} nmol/ml is equivalent to between 5 and 100 ng/ml and Dinbergs *et al* teach TGFβ at 15-30 ng/ml is effective for inducing formation of extracellular matrix by endothelial cell and smooth muscle cell without increasing cell proliferation.

(11) Response to Argument

Rejection of claims 1 and 6-9 under 35 U.S.C. § 103(a) over WO 94/23740 in view of Dinbergs

At page 6 of the Brief, Appellants argue that WO 94/23740 does not teach coupling growth factors to a polymeric scaffold.

Appellants' arguments have been fully considered but are not found to be persuasive. Claims 1 and 6-9 do not recite "coupling growth factors to a polymeric scaffold". The WO 94/23740 publication teaches a method for making a tissue engineering scaffold such as bone formation (See page 7, line 9-13, in particular) comprising coupling various matrix-enhancing molecules such as TGFβ or TGFβ2 to a polymer matrix such as polyethylene glycol having a molecule weight such as 5000 (M-S-PEG 5000) which is between 2000 and 6000 (See page 12, lines 12-14, PEG-TGF-β conjugates, rhTGF- TGF-β2 (PEG5000) bridging page 13, in particular). The WO 94/23740 publication further teaches that the reference matrix-enhancing molecule TGF-β is covalently coupled to tether or linking group such as hydroxysuccinimide to the scaffold such as the hydrophilic polymer such as polyethylene glycol (PEG5000) (See page 11, lines 10-28, in particular). The reference method is useful for stimulation of bone formation at a lower dose (See abstract, page Summery of invention, in particular). The WO 94/2370 publication teaches that the method results affect variable (See abstract, page 2, lines 11-15, in particular).

At page 7, first paragraph and item (1) in summary of the Brief, Appellants argue that WO 94/23740 does not mention using TGF-β to increase extracellular matrix production. The reference notes significant increases in proliferation of osteoblast-like cells (page 20, lines 7-22). The WO 94/23740 does not teach coupling to a matrix-enhancing molecule to a tissue engineering scaffold in an effective density to increase extracellular matrix production without increasing cellular proliferation.

Appellants' arguments have been fully considered but are not found to be persuasive. Claim 1 merely requires when the matrix enhancing molecule is TGFβ, the TGFβ is coupled to a matrix by a polymer tether having a molecular weight between 2000 and 6000 and is in a density between 1 and 100 ng TGF-β/ml and does not require the growth factor TGFβ be covalently coupled to a polymeric scaffold or cell bound to the scaffold.

The WO 94/23740 publication teaches a method for making a tissue engineering scaffold such as bone formation (See page 7, line 9-13, in particular) comprising coupling various matrix-enhancing molecules such as TGFβ or TGFβ2 to a polymer matrix such as polyethylene glycol having a molecule weight such as 5000 (M-S-PEG 5000) which is between 2000 and 6000 (See page 12, lines 12-14, PEG-TGF-β conjugates, rhTGF- TGF-β2 (PEG5000) bridging page 13, in particular). The WO 94/23740 publication further teaches that the reference matrix-enhancing molecule TGF-β are covalently coupled to tethers or linking group such as hydroxysuccinimide to the scaffold such as the hydrophilic polymer such as polyethylene glycol (PEG5000) (See page 11, lines 10-28, in particular). The reference method of making a tissue engineering scaffold comprises coupling TGFβ to a polymer which is useful for stimulation of bone formation at a lower dose (See abstract, page Summery of invention, in particular). The WO 94/2370 publication teaches that the method results affect variable (See abstract, page 2, in particular).

The claimed invention in claim 1 differs from the teachings of the reference only that the method for making a tissue engineering scaffold without increasing cellular proliferation and the TGF-β is in a density between 1 and 100 ng/ml.

Dinbergs *et al* teach a method for making a tissue engineering scaffold or formation of extracellular matrix by cells such as endothelial cells or smooth muscle cells seeded to the scaffold such as plastic of a 12-well tissue cultured plate. The reference method comprises coupling various matrix-enhancing molecule such as bFGF or TGFβ to a polymer such as alginate Heparin Sepharose microphere (See page 29823, Alginate/Heparin-Sepharose Microsphere Preparation and Growth Factor Incorporation, page 29823, column 2, bridging page 29824 column 1, in particular). The coupling of TGFβ to the polymer is at a concentration of 3 ng/sphere and 5-10 Evac TGFβ1 microspheres are placed in 1 ml, which is equivalent to 15-30 ng/ml and is within the claimed limit of a density between 1 and 100 ng TGFβ/ml (See page 29823, fourth paragraph, in particular). The reference concentration of TGFβ coupled to the polymer tether is *without* increasing cellular proliferation or cell number (See page 29825, Figure 3A (Endothelial cell) and 3B (smooth muscle cell), comparing filled circle to the open circle which is the control, in particular). Dinbergs *et al* further teach that TGFβ has been incorporated into scaffold or various biodegradable polymer matrix such as collagen, hydrogels such as alginate, hydron (hyaluronic acid) and polyethylene glycol polymers (See page 29827, column 2, first full paragraph, in particular). Dinbergs *et al* teach TGFβ is useful for eliciting extracellular matrix formation without increasing cellular proliferation for up to five days when coupling to

various polymer such as alginate hydrogel for a sustained release (See page 29825, Fig 3A, in particular). In fact, the *without* increasing cellular proliferation or cell number in smooth muscle cell as taught by Dinbergs *et al* is the same effect on the same cell type using the same TGFβ as disclosed on page 14, line 18-19 of instant specification.

At page 7 item (2) in the summary paragraph of the Brief, Appellants argue that WO 94/23740 does not teach an effective density of between 1 and 100 ng TGFβ/ml.

Appellants' arguments have been fully considered but are not found to be persuasive. The WO 94/2370 publication teaches that the method results affect variable which have been discussed supra.

Dinbergs *et al* teach a method for making a tissue engineering scaffold or formation of extracellular matrix by cells such as endothelial cells or smooth muscle cells seeded to the scaffold such as plastic of a 12-well tissue cultured plate. The reference method comprises coupling various matrix-enhancing molecule such as bFGF or TGFβ to a polymer such as alginate Heparin Sepharose microphere (See page 29823, Alginate/Heparin-Sepharose Microsphere Preparation and Growth Factor Incorporation, page 29823, column 2, bridging page 29824 column 1, in particular). The coupling of TGFβ to the polymer is at a concentration of 3 ng/sphere and 5-10 Evac TGFβ1 microspheres are placed in 1 ml, which is equivalent to 15-30 ng/ml and is within the claimed limit of a density between 1 and 100 ng TGFβ/ml (See page 29823, fourth paragraph, in particular). The reference concentration of TGFβ coupled to the polymer tether is *without* increasing cellular proliferation or cell number (See page 29825, Figure 3A (Endothelial cell) and 3B (smooth muscle cell), comparing filled circle to the open circle which is the control, in particular).

At page 7 item (3) in the summary paragraph of the Brief, Appellants argue that WO 94/23740 does not mention attaching cells to a polymeric scaffold (claim 2).

Appellants' argument with respect to claim 2 has been considered but is moot in view of the rejection of claim 2 has been withdrawn.

At page 7 item (4) in the summary paragraph of the Brief, Appellants argue that WO 94/23740 does not teach coupling matrix-enhancing molecules to tethers which are covalently bound to the scaffold (claim 6).

Appellants' arguments have been fully considered but are not found to be persuasive. The WO 94/23740 publication teaches a matrix-enhancing molecule such as TGF-β and the reference TGF-β is covalently coupled to tethers or linking group such as hydroxysuccinimide to the matrix such as the hydrophilic polymer such as 5000 (M-S-PEG 5000) which is between 2000 and 6000 (See page 12, lines 12-14, PEG-TGF-β conjugates, rhTGF- TGF-β2 (PEG5000) bridging page 13, page 11, lines 10-28, in particular).

At page 7 item (5) in the summary paragraph of the Brief, Appellants argue that WO 94/23740 does not teach a tissue engineering scaffold that is a hydrogel (claim 7), wherein the hydrogel may be alginate, collagen, hyauronic acid and polyethylene glycol polymers (claim 8).

Appellants' arguments have been fully considered but are not found to be persuasive. Dinbergs *et al* teach various tissue engineering scaffold such as biodegradable polymer matrix such as collagen, hydrogels such as alginate, hydron (hyaluronic acid) and polyethylene glycol polymers (See page 29827, column 2, first full paragraph, in particular). In contrast to appellants' assertion that the WO 94/23740 publication does not teach polymer is polyethylene glycol, the WO 94/23740 publication teaches a method for making a tissue engineering scaffold such as bone formation (See page 7, line 9-13, in particular) comprising coupling various matrix-enhancing molecules such as TGFβ or TGFβ2 to a polymer matrix such as polyethylene glycol having a molecule weight such as 5000 (M-S-PEG 5000) which is between 2000 and 6000 (See page 12, lines 12-14, PEG-TGF-β conjugates, rhTGF- TGF-β2 (PEG5000) bridging page 13, in particular).

At page 7 item (6) in the summary paragraph of the Brief, Appellants argue that WO 94/23740 does not teach coupling TGFβ to the hydrogel in a concentration of 1 to 100 ng TGFβ/ml (claim 9).

Appellants' arguments have been fully considered but are not found to be persuasive. Dinbergs *et al* teach coupling of TGFβ to a polymer such as alginate Heparin Sepharose microphere at a concentration of 3 ng/sphere; 5-10 Evac TGFβ1 microspheres are placed in 1 ml, which is equivalent to 15-30 ng/ml; the reference concentration is within the claimed limit of a density between 1 and 100 ng TGFβ/ml which is equivalent to between 4×10^{-6} to 4×10^{-3} nmol/ml (See page 29823, fourth paragraph, in particular).

At page 8 first paragraph of the Brief, Appellants argue that Dinbergs et al teach growth factors are not coupled to a polymeric scaffold, but instead are encapsulated by a polymeric matrix and released as soluble growth factors.

Appellants' arguments have been fully considered but are not found to be persuasive. Claim 1 merely requires when the matrix enhancing molecule is TGFβ, the TGFβ is coupled to a matrix by a polymer tether having a molecular weight between 2000 and 6000 and is in a density between 1 and 100 ng TGF-β/ml and does not require the growth factor TGFβ be covalently coupled to a polymeric scaffold or cell bound to the scaffold.

At page 8 second paragraph of the Brief, Appellants argue that Dinbergs does not discuss or suggest that extracellular matrix production may be enhanced without cellular proliferation. While Dinbergs makes mention that TGFβ mediates accumulation of extracellular matrix (page 29822, col 2, last paragraph), and later shows that sustained-release TGFβ is a poor inhibitor of cellular proliferation (Figure 3A and 3B), this is no way suggests that matrix accumulation may be stimulated independently of cellular proliferation.

Appellants' arguments have been fully considered but are not found to be persuasive. It is noted that none of the rejected claims recite the claimed method *enhances* extracellular matrix production without increasing cellular proliferation. Further, the claimed method does not require *inhibition* of cellular proliferation. Claim 1 merely requires when the matrix enhancing molecule is TGFβ, the TGFβ is coupled to a matrix by a polymer tether having a molecular weight between 2000 and 6000 and is in a density between 1 and 100 ng TGF-β/ml and does not require the growth factor TGFβ be covalently coupled to a polymeric scaffold or cell bound to the scaffold.

Dinbergs *et al* teach a method for making a tissue engineering scaffold by coupling TGFβ to a polymer tether such as alginate Heparin Sepharose microphere at a concentration of 3 ng/sphere; 5-10 Evac TGFβ1 microspheres are placed in 1 ml, which is equivalent to 15-30 ng/ml; the reference concentration is within the claimed limit of a density between 1 and 100 ng TGFβ/ml (See page 29823, fourth paragraph, in particular) and is without increasing cellular proliferation or cell number (See page 29825, Figure 3A (Endothelial cell) and 3B (smooth muscle cell), comparing filled circle to the open circle which is the control, in particular).

At page 9 first paragraph of the Brief, Appellants argue that Dinbergs states that the end concentration is "3ng of TGFβ/microsphere" (page 29823, EVAc Microsphere Preparation and Growth Factor Incorporation), assuming the volume of the microspheres is far less than 1 ml, it is clear that the concentration does not fall within the 1-10ng/ml concentration as alleged by the Examiner, nor in the 1-100ng/ml concentration as claimed by Appellants.

Appellants' arguments have been fully considered but are not found to be persuasive. It is mutually agreed that Dinbergs et al teach "3ng of TGFβ/microsphere" (page 29823, EVAc Microsphere Preparation and Growth Factor Incorporation). Dinbergs et al further teach that 5-10 Evac TGFβ1 microspheres are placed in 1 ml, which is equivalent to 15-30 ng/ml (3ng multiply by 5 or 10 microsphere/ml) and is within the claimed limit of a density between 1 and 100 ng TGFβ/ml (See page 29823, fourth paragraph, in particular). Dinbergs et al also teach TGFβ (without conjugating to the microsphere) at a concentration of 1-10 ng/ml.

At page 9 second paragraph item (1) of the Brief, Appellants argue that Dinbergs does not disclose coupling a matrix-enhancing molecule to a tissue engineering scaffold in an effective density to increase extracellular matrix production without increasing cellular proliferation (claim 1).

Appellants' arguments have been fully considered but are not found to be persuasive. It noted that claim 1 does not recite coupling a matrix-enhancing molecule to a tissue engineering scaffold in an effective density to *increase* extracellular matrix production without increasing cellular proliferation (claim 1). Claim 1 recites a method for making a tissue engineering scaffold for inducing formation of extracellular matrix by cells bound to the scaffold comprising coupling matrix-enhancing molecules to the scaffold in an effective density to elicit production of extracellular matrix without increasing cellular proliferation, wherein *when* the matrix-enhancing molecules are TGF-β, the TGF-β is coupled to the matrix by a polymer tether having a molecular weight between 2000 and 6000 and is in a density between 1 and 100 ng TGF-β/ml. The TGF-β simply tethers to the polymer matrix having a molecular weight between 2000 and 6000 and in a density between 1 and 100 ng TGF-β/ml. Dinbergs *et al* teach a method for making a tissue engineering scaffold by coupling TGFβ to a polymer tether such as alginate Heparin Sepharose microphere at a concentration of 3 ng/sphere; 5-10 Evac TGFβ1 microspheres are placed in 1 ml, which is equivalent to 15-30 ng/ml; the reference concentration is within the claimed limit of a density between 1 and 100 ng TGFβ/ml (See page 29823, fourth paragraph, in particular) and is without increasing cellular proliferation or cell number (See page 29825, Figure 3A (Endothelial

cell) and 3B (smooth muscle cell), comparing filled circle to the open circle which is the control, in particular).

At page 9 second paragraph item (2) of the Brief, Appellants argue that the WO94/23740 does not disclose an effective density of between 1 and 100 ng TGFβ/ml.

Appellants' arguments have been fully considered but are not found to be persuasive. The rejection would have been under USC 102(b) if the WO94/23740 discloses an effective density of between 1 and 100ng TGFβ/ml. The WO 94/2370 publication teaches that the method results affect variable which have been discussed supra.

Dinbergs *et al* teach a method for making a tissue engineering scaffold or formation of extracellular matrix by cells such as endothelial cells or smooth muscle cells seeded to the scaffold such as plastic of a 12-well tissue cultured plate. The reference method comprises coupling various matrix-enhancing molecule such as bFGF or TGFβ to a polymer such as alginate Heparin Sepharose microphere (See page 29823, Alginate/Heparin-Sepharose Microsphere Preparation and Growth Factor Incorporation, page 29823, column 2, bridging page 29824 column 1, in particular). The coupling of TGFβ to the polymer is at a concentration of 3 ng/sphere and 5-10 Evac TGFβ1 microspheres are placed in 1 ml, which is equivalent to 15-30 ng/ml and is within the claimed limit of a density between 1 and 100 ng TGFβ/ml (See page 29823, fourth paragraph, in particular). The reference concentration of TGFβ coupled to the polymer tether is *without* increasing cellular proliferation or cell number (See page 29825, Figure 3A (Endothelial cell) and 3B (smooth muscle cell), comparing filled circle to the open circle which is the control, in particular).

At page 9 item (3) of the Brief, Appellants argue that Dinbergs makes no mention of attaching cells to a polymeric scaffold (claim 2).

Appellants' argument with respect to claim 2 has been considered but is moot in view of the rejection has been withdrawn.

At page 9 item (4) of the Brief, Appellants argue that Dinbergs does not disclose coupling matrix-enhancing molecules to tethers which are covalently bound to the scaffold (claim 6).

Appellants' arguments have been fully considered but are not found to be persuasive. The WO 94/23740 publication teaches that the reference matrix-enhancing molecule TGF-β are

either directly or indirectly covalently coupled to tethers or linking group such as hydroxysuccinimide to the scaffold such as the hydrophilic polymer such as polyethylene glycol (PEG5000) (See page 11, lines 10-28, in particular).

At page 10 item (5) of the Brief, Appellants argue that Dinbergs does not disclose a tissue engineering scaffold that is a hydrogel (claim 7), wherein the hydrogel may be alginate, collagen, hyaluronic acid and polyethylene glycol polymers (claim 8).

Appellants' arguments have been fully considered but are not found to be persuasive. Dinbergs *et al* teach that TGFβ can be incorporated into scaffold or various biodegradable polymer matrix such as collagen, hydrogels such as alginate, hydron (hyaluronic acid) and polyethylene glycol polymers (See page 29827, column 2, first full paragraph, in particular). Further, the WO 94/23740 publication teaches a method for making a tissue engineering scaffold such as bone formation (See page 7, line 9-13, in particular) comprising coupling various matrix-enhancing molecules such as TGFβ or TGFβ2 to a polymer matrix such as polyethylene glycol having a molecule weight such as 5000 (M-S-PEG 5000) which is between 2000 and 6000 (See page 12, lines 12-14, PEG-TGF-β conjugates, rhTGF- TGF-β2 (PEG5000) bridging page 13, in particular).

At page 10 item (6) of the Brief, Appellants argue that Dinbergs does not disclose coupling TGF-β to the hydrogel in a concentration of 1 to 100 ng TGF-β/ ml.

Appellants' arguments have been fully considered but are not found to be persuasive. In contrast to appellants' assertion that Dinbergs does not disclose coupling TGF-β to the hydrogel in a concentration of 1 to 100 ng TGF-β/ ml, Dinbergs et al teach coupling of the reference TGF-β to the hydrogel that is made of a polymer such as alginate Heparin Sepharose microphere (See page 29823, Alginate/Heparin-Sepharose Microsphere Preparation and Growth Factor Incorporation, page 29823, column 2, bridging page 29824 column 1, in particular). The coupling of TGFβ to the polymer is at a concentration of 3 ng/sphere and 5-10 Evac TGFβ1 microspheres are placed in 1 ml, which is equivalent to 15-30 ng/ml and is within the claimed limit of a density between 1 and 100 ng TGFβ/ml (See page 29823, fourth paragraph, in particular). Dinbergs *et al* further teach that it is known at the time the invention was made that TGFβ has been incorporated into scaffold such as biodegradable polymer matrix made of

collagen, hydrogels such as alginate, hydron (hyaluronic acid) and polyethylene glycol polymers (See page 29827, column 2, first full paragraph, in particular).

At page 10 of the Brief, Appellants argue that the combination of WO94/23740 with Dinbergs does not make obvious claim 1 since WO 94/23740 discloses the use of *soluble* polymer-growth factor conjugates. Dinbergs teaches encapsulated growth factors which are released as soluble growth factors. And neither reference discloses coupling TGF-β in an effective density to *enhance extracellular matrix production* without increasing cell proliferation.

Appellants' arguments have been fully considered but are not found to be persuasive. The polymer-growth factor conjugate such as TGF-β coupled to polyethylene glycol taught by the WO94/23740 is identical to the polymer-growth factor conjugate as disclosed on page 9 line 24-25 of the specification. Dinbergs *et al* teach that it is known at the time the invention was made that TGFβ has been incorporated into scaffold such as biodegradable polymer matrix made of collagen, hydrogels such as alginate, hydron (hyaluronic acid) and polyethylene glycol polymers (See page 29827, column 2, first full paragraph, see instant claims 7-8, in particular). Further, none of the rejected claims recite coupling TGF-β in an effective density to *enhance extracellular matrix production* without increasing cell proliferation. Given the claimed method steps using the same matrix-enhancing molecule and the same polymer as that of the prior arts, it would have been obvious that the results of the claimed method are the same as that of the prior arts.

At page 10 of the Brief, Appellants argue that the combination of WO94/23740 with Dinbergs does not make obvious claim 2 since neither reference discloses the additional limitation of attaching cells to the scaffold.

In response, Appellants' argument with respect to claim 2 are moot in view of the rejection of claim 2 has been withdrawn.

At page 10 of the Brief, Appellants argue that the combination of WO94/23740 with Dinbergs does not make obvious claims 3 and 5 since neither reference discloses the coupling of matrix-enhancing molecules such as angiotensin II and ascorbic acid to a polymeric scaffold.

Appellants' arguments have been fully considered but are not found to be persuasive. Claims 3 and 5 are withdrawn from consideration as NOT directed to TGFβ under examination and thus Claims 3 and 5 are not on appeal.

At page 10 of the Brief, Appellants argue that the combination of WO94/23740 with Dinbergs does not make obvious claim 4 since neither reference discloses the coupling of matrix-enhancing molecules such as insulin-like growth factors to a polymeric scaffold.

Appellants' arguments have been fully considered but are not found to be persuasive. Claim 4 is withdrawn from consideration as NOT directed to TGFβ under examination and thus Claim 4 is not on appeal.

At page 11 of the Brief, Appellants argue that the combination of WO94/23740 with Dinbergs does not make obvious claim 6 since neither reference discloses coupling matrix-enhancing molecules to tethers which are coupled to the scaffold.

Appellants' arguments have been fully considered but are not found to be persuasive. The WO 94/23740 publication teaches matrix-enhancing molecules such as TGF-β are covalently coupled either directly or indirectly to tethers or linking group such as hydroxysuccinimide to the scaffold/matrix such as the hydrophilic polymer such as polyethylene glycol (PEG5000) (See page 11, lines 10-28, in particular).

At page 11 of the Brief, Appellants argue that the combination of WO94/23740 with Dinbergs does not make obvious claims 7-9 since neither reference discloses a polymeric scaffold, much less a polymeric scaffold that is a hydrogel.

Appellants' arguments have been fully considered but are not found to be persuasive. In contrast to appellants' argument that neither reference discloses a polymeric scaffold, Dinbergs *et al* teach that it is known at the time the invention was made that TGFβ has been incorporated into scaffold such as biodegradable polymer matrix made of collagen, hydrogels such as alginate, hydron (hyaluronic acid) and polyethylene glycol polymers (See page 29827, column 2, first full paragraph, in particular).

Rejection of claims 1-2 and 6-9 under 35 U.S.C. § 103(a) over WO 96/27657 in view of Dinbergs

At page 11 of the Brief, Appellants argue that the WO 96/27657 teaches localized growth factors result in a higher rate of cell growth and are effective at lower dosages as compared to soluble growth factor (see page 5, line 27, claims 1, 13 and 31). The reference does not teach coupling matrix-enhancing molecules to a polymeric scaffold to increase extracellular matrix production.

Appellants' arguments have been fully considered but are not found to be persuasive. In contrast to appellants' argument that the WO 96/27657 publication teaches localized growth factors result in a higher rate of cell growth and are effective at lower dosages as compared to soluble growth factor (see page 5, line 27, claims 1, 13 and 31), it is noted that the definition of cell growth as taught by the WO96/27657 is to enhance the long term stability of differentiated mammalian cells or to enhance the biological response of the cell (see page 4, summary of the invention, page 1, line 12-19, I particular). The WO 96/27657 does not teach cell growth is equivalent to increase in cell number or cellular proliferation. In contrast to appellants' argument that the WO 96/27657 publication does not teach coupling matrix-enhancing molecules to a polymeric scaffold, the WO 96/27657 publication teaches a method for making a tissue engineering scaffold comprising coupling various matrix-enhancing molecules such as TGFβ (see page 10, claim 25 of WO 96/27657 publication, in particular) flexibly linked or tethered (See page 6, line 11, page 12, Attachment methods, in particular) using carbodiimides as cross-linker to substrate such as polymer matrix such as bottles, dishes, fibers, shaped polymers, particles, microparticles (See page 9, lines 25-26, in particular), tissue regeneration devices such as collagen, or polyethylene oxide, alginate, (See page 17, lines 1-12, in particular). The reference polymer tether has a molecular weight 3000-12,000 (See page 7, line 15, in particular). The reference method further attaches cells to the reference scaffold (See page 16, line 7, in particular) for constructing tissue regeneration such as production of extracellular matrix proteins such as collagen (See page 17, line 1-4, in particular). In response to Appellants' arguments that the method "increase extracellular matrix production", it is noted that none of the claims recite "*increase* extracellular matrix production". The WO 96/27657 publication teaches growth factors that are important for extracellular matrix production such as TGFβ (See page 2, lines 5-13, in particular).

At page 12 first paragraph of the Brief, Appellants argue that the WO 96/27657 does not disclose an effective density of 1 to 100 ng TGFβ/ml. The reference also fails to disclose a tissue engineering scaffold that is a hydrogel.

Appellants' arguments have been fully considered but are not found to be persuasive. The rejections of claims 1-2, and 6-9 would have been under 35 USC 102(b) if the WO 96/27657 publication teaches effective density of 1 to 100 ng TGFβ/ml.

The WO 96/27657 publication teaches a method for making a tissue engineering scaffold comprising coupling various matrix-enhancing molecules such as TGFβ (see page 10, claim 25 of WO 96/27657 publication, in particular) flexibly linked or tethered (See page 6, line 11, page 12, Attachment methods, in particular) using carbodiimides as cross-linker to substrate such as polymer matrix such as bottles, dishes, fibers, shaped polymers, particles, microparticles (See page 9, lines 25-26, in particular), tissue regeneration devices such as collagen, or polyethylene oxide, alginate, (See page 17, lines 1-12, in particular). The reference polymer tether has a molecular weight 3000-12,000 (See page 7, line 15, in particular). The reference method further attaches cells to the reference scaffold (See page 16, line 7, in particular) for constructing tissue regeneration such as production of extracellular matrix proteins such as collagen (See page 17, line 1-4, in particular). The WO 96/27657 publication teaches the growth factor is localized to desired target cell population and significantly less growth factor is needed to exert a biologic response (See abstract, in particular).

The claimed invention in claim 1 differs from the teachings of the reference only that the method for making a tissue engineering scaffold without increasing cellular proliferation and the TGF-β is in a density between 1 and 100 ng/ml.

Dinbergs *et al* teach a method for making a tissue engineering scaffold or formation of extracellular matrix by cells such as endothelial cells or smooth muscle cells seeded to the scaffold such as plastic of a 12-well tissue cultured plate. The reference method comprises coupling various matrix-enhancing molecule such as bFGF or TGFβ to a polymer such as alginate Heparin Sepharose microphere (See page 29823, Alginate/Heparin-Sepharose Microsphere Preparation and Growth Factor Incorporation, page 29823, column 2, bridging page 29824 column 1, in particular). The coupling of TGFβ to the polymer is at a concentration of 3 ng/sphere and 5-10 Evac TGFβ1 microspheres are placed in 1 ml, which is equivalent to 15-30 ng/ml and is within the claimed limit of a density between 1 and 100 ng TGFβ/ml (See page 29823, fourth paragraph, in particular). The reference concentration of TGFβ coupled to the

polymer tether is *without* increasing cellular proliferation or cell number (See page 29825, Figure 3A (Endothelial cell) and 3B (smooth muscle cell), comparing filled circle to the open circle which is the control, in particular). Dinbergs *et al* further teach that TGFβ has been incorporated into scaffold or various biodegradable polymer matrix such as collagen, hydrogels such as alginate, hydron (hyaluronic acid) and polyethylene glycol polymers (See page 29827, column 2, first full paragraph, in particular). Dinbergs *et al* teach TGFβ is useful for eliciting extracellular matrix formation without increasing cellular proliferation for up to five days when coupling to various polymer such as alginate hydrogel for a sustained release (See page 29825, Fig 3A, in particular).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to use TGFβ at a concentration of 15-30 ng/ml as taught by Dinbergs *et al* for a method of making a tissue engineering scaffold for inducing formation of extracellular matrix by cells bound to the scaffold without increasing cellular proliferation as taught by the WO 96/27657 publication and Dinbergs *et al.* From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention.

One having ordinary skill in the art would have been motivated to do this because the WO 96/27657 publication teaches that when growth factor is localized to desired target cell, significantly less growth factor is needed to exert a biologic response (See abstract, in particular). Dinbergs *et al* teach that TGFβ coupling to polymer at a concentration of 15-30 ng/ml is useful for eliciting extracellular matrix formation without increasing cellular proliferation or cell number (See page 29825, Figures3A (Endothelial cell) and 3B (smooth muscle cell), comparing filled circle to the open circle which is the control, in particular). Claim 9 is included in this rejection because between 4×10^{-6} to 4×10^{-3} nmol/ml is equivalent to between 5 and 100 ng/ml and Dinbergs *et al* teach TGFβ at 15-30 ng/ml is effective for inducing formation of extracellular matrix by endothelial cell and smooth muscle cell without increasing cell proliferation.

At page 12 second paragraph of the Brief, Appellants argue that there is no suggestion in either reference to incorporate the teachings of the other. The claims of WO 96/27657 are directed to methods and compositions for stimulating eukaryotic cell growth. Dinbergs teaches TGF-β as an inhibitor of cellular proliferation, not promoter of proliferation. The WO96/27657 describes the use of immobilized TGF-β, while Dinbergs discloses soluble TGF-β. Dinbergs clearly concludes that soluble, bolus administration of TGF-β is the preferred method of administration.

Appellants' arguments have been fully considered but are not found to be persuasive. The claims of WO 96/27657 are directed to methods and compositions for stimulating eukaryotic cell growth in terms of function and not cell number (cellular proliferation) as asserted by Appellants. It is noted that none of the claims of the WO96/27657 recites the reference method and composition stimulates cellular proliferation. The WO96/27657 publication teaches known methods and compositions for culturing cells and implanting them into the body by having tethered growth effector molecules such as TGFβ to the microspheres to improve their usefulness at the time the invention was made (See page 16, lines 9-14, in particular). In contrast to Appellants' assertion that Dinbergs teaches TGF-β as an inhibitor of cellular proliferation, not promoter of proliferation, Dinbergs et al do not teach TGF-β as an inhibitor of cellular proliferation. Dinbergs et al teach that coupling of TGFβ to the polymer is at a concentration of 3 ng/sphere and 5-10 Evac TGFβ1 microspheres are placed in 1 ml, which is equivalent to 15-30 ng/ml and is within the claimed limit of a density between 1 and 100 ng TGFβ/ml (See page 29823, fourth paragraph, in particular) is *without* increasing cellular proliferation or cell number (See page 29825, Figure 3A (Endothelial cell) and 3B (smooth muscle cell), comparing filled circle to the open circle which is the control, in particular), which is consistent with the claimed invention.

At paragraph bridging pages 12 and 13 of the Brief, Appellants argue that neither WO96/27657 nor Dinbergs disclose the benefits of enhancing extracellular matrix formation without increasing cellular proliferation. Nor do the references disclose coupling TGF-β to a polymeric scaffold in an effective density (1-100 ng/ml) to enhance cellular proliferation.

Appellants' arguments have been fully considered but are not found to be persuasive. It is noted that none of the claims are drawn to a method of enhancing extracellular matrix formation without increasing cellular proliferation. Further, the reason or motivation to modify

the reference may often suggest what the inventor has done, but for a different purpose or to solve a different problem. It is not necessary that the prior art suggest the combination to achieve the same advantage or result discovered by applicant. In re Linter, 458 F.2d 1013, 173 USPQ 560 (CCPA 1972) (discussed below); In re Dillon, 919 F.2d 688, 16 USPQ2d 1897 (Fed. Cir. 1990), cert. denied, 500 U.S. 904 (1991) (discussed below). Although Ex parte Levengood, 28 USPQ2d 1300, 1302 (Bd. Pat. App. & Inter. 1993) states that obviousness cannot be established by combining references "without also providing evidence of the motivating force which would impel one skilled in the art to do what the patent applicant has done " (emphasis added), reading the quotation in context it is clear that while there must be motivation to make the claimed invention, there is no requirement that the prior art provide the same reason as the applicant to make the claimed invention. The motivation to combine the reference is clear from the teaching of the WO 96/27657 publication that when TGFβ is tethered to the matrix, significantly less growth factor is needed to exert a biologic response (See abstract, in particular). Dinbergs et al teach that at a concentration of 3 ng/sphere and 5-10 Evac TGFβ1 microspheres are placed in 1 ml, which is equivalent to 15-30 ng/ml and is within the claimed limit of a density between 1 and 100 ng TGFβ/ml (See page 29823, fourth paragraph, in particular) is *without* increasing cellular proliferation or cell number (See page 29825, Figure 3A (Endothelial cell) and 3B (smooth muscle cell), comparing filled circle to the open circle which is the control, in particular), which is consistent with the claimed invention.

At page 13 second paragraph of the Brief, Appellants argue that the combination of WO96/27657 and Dinbergs does not make obvious claims 3 and 5 since neither reference discloses the coupling of matrix-enhancing molecules such as angiotensin II and ascorbic acid to a polymeric scaffold.

Appellants' arguments have been fully considered but are not found to be persuasive. Claims 3 and 5 are withdrawn from consideration as NOT directed to TGFβ under examination and thus Claims 3 and 5 are not on appeal.

At page 13 third paragraph of the Brief, Appellants argue that the combination of WO96/27657 and Dinbergs does not make obvious claims 7-9 since neither reference teaches the additional limitations of claims 7-9, relating to a hydrogel scaffold.

Appellants' arguments have been fully considered but are not found to be persuasive. In contrast to appellants' argument that neither reference discloses a polymeric scaffold that is a hydrogel, Dinbergs *et al* teach that it is known at the time the invention was made that TGFβ has been incorporated into scaffold such as biodegradable polymer matrix made of collagen, hydrogels such as alginate, hydron (hyaluronic acid) and polyethylene glycol polymers (See page 29827, column 2, first full paragraph, in particular). The WO 96/27657 publication teaches the scaffold for constructing tissue regeneration devices such as extracellular matrix proteins such as collagen, polylactic acid, polyglycolic acid, biocompatible polymers (see page 17, claims 16-23 of WO 96/27657, in particular). In fact, instant specification on page 5 discloses that the scaffold is formed by synthetic or natural polymers; the scaffold may be biodegradable such as polylactic acid, polyglycolic acid, and natural polymers include collagen, hyaluronic acid and albumin or non-degradable.

At page 14 of the Brief, Appellants argue that the claimed method produced unexpected results in view of the prior art. Appellants' combination of immobilized growth factor technology and the inhibitory properties of TGF-β result in an unexpectedly improved method of enhancing extracellular matrix formation without an increase in cell proliferation. WO 94/23740 notes significant increases in proliferation of osteoblast-like cell population. WO 96/27657 is directed to methods and compositions for stimulating eukaryotic cell growth (see abstract and claims 1, 13 and 31) and Dinbergs argues that TGF-β1 should not be released in a sustained manner because of its inability of reduced efficacy in inhibiting cell growth, citing an 18.0 fold increase in endothelial number over the original plating density and a 115.o fold increase for smooth muscle cells (see page 29825, col 1, last paragraph, bridging col 2). In many tissue engineering application it is important to avoid undesired enhancement of cell growth. For example, in vascular tissue engineering, over-proliferation of the smooth muscle cells can lead to a failure of the tissue engineering construct due to luminal narrowing. This is unexpected in light of the prior a11: WO 94/23740 discloses a soluble polymer-conjugated growth factor which increases cellular proliferation; WO 96/26757 discloses growth factors coupled to scaffolds which increase cellular proliferation; and Dinbergs teaches TGF-β sustain-released from microspheres is unable to effectively inhibit cell proliferation.

Appellants' arguments have been fully considered but are not found to be persuasive. Appellants' reliance on unexpected results does not overcome clear and convincing evidence of

obviousness. Also see Richardson-Vicks Inc. v. Upjohn Co., 44 USPQ2d 1181 (CAFC 1997). Appellants argued that the combination of immobilized growth factor and the inhibitory properties of TGF-β unexpectedly *improved* the method of enhancing extracellular matrix formation without an increase in cell proliferation. However, none of the claims recite a method of *enhancing* extracellular matrix formation without an increase in cell proliferation or a method of vascular tissue engineering without an increase in cell proliferation. Further, the specification discloses that the production of collagen, which is one of the extracellular matrix, by smooth muscle cells (SMC) using TGFβ tethered to polyethylene glycol (acryloyl-PEG-TGFβ) is not significantly different compared to unmodified soluble TGFβ (See Figure 3, overlapping standard deviation bar, page 13, lines 24-27, in particular). However, more hydroxyproline and thus more collagen are produced by SMCs grown in the presence of either soluble or tethered TGFβ than when no TGFβ is present (Figure 3, page in particular).

In response to Appellants' argument that the claimed method *without* an increase in cell proliferation, the term "*without* an increase in cell proliferation" as recited in claim 1 is not equivalent to "inhibit cell proliferation" as argued by appellants. Dinbergs *et al* teach a method for making a tissue engineering scaffold or formation of extracellular matrix by cells such as endothelial cells or smooth muscle cells seeded to the scaffold such as plastic of a 12-well tissue cultured plate. The reference method comprises coupling various matrix-enhancing molecule such as bFGF or TGFβ to a polymer such as alginate Heparin Sepharose microphere (See page 29823, Alginate/Heparin-Sepharose Microsphere Preparation and Growth Factor Incorporation, page 29823, column 2, bridging page 29824 column 1, in particular). The reference TGFβ is coupled to the reference polymer at a concentration of 3 ng/sphere and 5-10 Evac TGFβ1 microspheres are placed in 1 ml, which is equivalent to 15-30 ng/ml and is within the claimed limit of a density between 1 and 100 ng TGFβ/ml (See page 29823, fourth paragraph, in particular). The reference concentration of TGFβ coupled to the polymer tether is *without* increasing cellular proliferation or cell number (See page 29825, Figure 3A (Endothelial cell) and 3B (smooth muscle cell), comparing filled circle to the open circle which is the control, in particular).

In response to appellants' argument that the WO 94/23740 notes significant increases in proliferation of osteoblast-like cell population, the WO 94/2370 publication teaches that the method results affect variable (See abstract, page 2, lines 11-15, in particular). Claim 1 merely requires when the matrix enhancing molecule is TGFβ, the TGFβ is coupled to a matrix by a polymer tether having a molecular weight between 2000 and 6000 and is in a density between 1 and 100 ng TGF-β/ml and does not require the growth factor TGFβ be covalently coupled to a polymeric scaffold or cell bound to the scaffold. The WO 94/23740 publication teaches a method for making a tissue engineering scaffold such as bone formation (See page 7, line 9-13, in particular) comprising coupling various matrix-enhancing molecules such as TGFβ or TGFβ2 to a polymer matrix such as polyethylene glycol having a molecule weight such as 5000 (M-S-PEG 5000) which is between 2000 and 6000 (See page 12, lines 12-14, PEG-TGF-β conjugates, rhTGF- TGF-β2 (PEG5000) bridging page 13, in particular). The WO 94/23740 publication further teaches that the reference matrix-enhancing molecule TGF-β are covalently coupled to tethers or linking group such as hydroxysuccinimide to the scaffold such as the hydrophilic polymer such as polyethylene glycol (PEG5000) (See page 11, lines 10-28, in particular). The reference method of making a tissue engineering scaffold comprises coupling TGFβ to a polymer which is useful for stimulation of bone formation at a lower dose (See abstract, page Summery of invention, in particular). The WO 94/2370 publication teaches that the method results affect variable (See abstract, in particular). WO 94/2370 publication teaches that peptide from the TGFβ family regulates both cell growth and cell differentiation depending upon the particular cell type (page 2, line 11-15, in particular).

The claimed invention in claim 1 differs from the teachings of the reference only that the method for making a tissue engineering scaffold without increasing cellular proliferation and the TGF-β is in a density between 1 and 100 ng/ml.

Dinbergs *et al* teach a method for making a tissue engineering scaffold or formation of extracellular matrix by cells such as endothelial cells or smooth muscle cells seeded to the scaffold such as plastic of a 12-well tissue cultured plate. The reference method comprises coupling various matrix-enhancing molecule such as bFGF or TGFβ to a polymer such as alginate Heparin Sepharose microphere (See page 29823, Alginate/Heparin-Sepharose Microsphere Preparation and Growth Factor Incorporation, page 29823, column 2, bridging page 29824 column 1, in particular). The reference TGFβ is coupled to the reference polymer at a concentration of 3 ng/sphere and 5-10 Evac TGFβ1 microspheres are placed in 1 ml, which is

equivalent to 15-30 ng/ml and is within the claimed limit of a density between 1 and 100 ng TGFβ/ml (See page 29823, fourth paragraph, in particular). The reference concentration of TGFβ coupled to the polymer tether is *without* increasing cellular proliferation or cell number (See page 29825, Figure 3A (Endothelial cell) and 3B (smooth muscle cell), comparing filled circle to the open circle which is the control, in particular). Dinbergs *et al* further teach that TGFβ has been incorporated into scaffold or various biodegradable polymer matrix such as collagen, hydrogels such as alginate, hydron (hyaluronic acid) and polyethylene glycol polymers (See page 29827, column 2, first full paragraph, in particular). Dinbergs *et al* teach TGFβ is useful for eliciting extracellular matrix formation without increasing cellular proliferation for up to five days when coupling to various polymer such as alginate hydrogel for a sustained release (See page 29825, Fig 3A, in particular). In fact, the *without* increasing cellular proliferation or cell number in smooth muscle cell as taught by Dinbergs *et al* is the same effect on the same cell type using the same TGFβ as disclosed on page 14, line 18-19 of instant specification.

In response to appellants' argument that the WO 96/27657 teaches methods and compositions for stimulating eukaryotic cell growth and Dinbergs argues that TGFβ should not be released in a sustained manner because of its' inability of reduced efficacy in inhibiting cell growth, it is noted that none of the claims of the WO96/27657 recites the reference method and composition stimulates cellular proliferation. Claim 1 merely requires TGFβ coupled to a polymer tether having a molecular weight between 2000 and 6000 and is in a density between 1 and 100 ng TGF-β/ml and does not require the TGFβ covalently coupled to tethers which are covalently coupled to the scaffold or cell bound to the scaffold.

The WO 96/27657 publication teaches a method for making a tissue engineering scaffold comprising coupling various matrix-enhancing molecules such as TGFβ (see page 10, claim 25 of WO 96/27657 publication, in particular) flexibly linked or tethered (See page 6, line 11, page 12, Attachment methods, in particular) using carbodiimides as cross-linker to substrate such as polymer matrix such as bottles, dishes, fibers, shaped polymers, particles, microparticles (See page 9, lines 25-26, in particular), tissue regeneration devices such as collagen, or polyethylene oxide, alginate, (See page 17, lines 1-12, in particular). The reference polymer tether has a molecular weight 3000-12,000 (See page 7, line 15, in particular). The reference method further attaches cells to the reference scaffold (See page 16, line 7, in particular) for constructing tissue regeneration such as production of extracellular matrix proteins such as collagen (See page 17, line 1-4, in particular). The WO 96/27657 publication teaches the growth factor is localized to

desired target cell population and significantly less growth factor is needed to exert a biologic response (See abstract, in particular).

The claimed invention in claim 1 differs from the teachings of the reference only that the method for making a tissue engineering scaffold wherein the method elicits production of extracellular matrix without increasing cellular proliferation and the TGF-β is in a density between 1 and 100 ng/ml.

The claimed invention in claim 9 differs from the reference only that the method wherein the matrix enhancing molecules are TGF-β is in a concentration between about 4×10^{-6} to 4×10^{-3} nmol/ml.

Dinbergs *et al* teach a method for making a tissue engineering scaffold or formation of extracellular matrix by cells such as endothelial cells or smooth muscle cells seeded to the scaffold such as plastic of a 12-well tissue cultured plate. The reference method comprises coupling various matrix-enhancing molecule such as bFGF or TGFβ to a polymer such as alginate Heparin Sepharose microphere (See page 29823, Alginate/Heparin-Sepharose Microsphere Preparation and Growth Factor Incorporation, page 29823, column 2, bridging page 29824 column 1, in particular). The coupling of TGFβ to the polymer is at a concentration of 3 ng/sphere and 5-10 Evac TGFβ1 microspheres are placed in 1 ml, which is equivalent to 15-30 ng/ml and is within the claimed limit of a density between 1 and 100 ng TGFβ/ml (See page 29823, fourth paragraph, in particular). The reference concentration of TGFβ coupled to the polymer tether is *without* increasing cellular proliferation or cell number (See page 29825, Figure 3A (Endothelial cell) and 3B (smooth muscle cell), comparing filled circle to the open circle which is the control, in particular). Dinbergs *et al* further teach that TGFβ has been incorporated into scaffold or various biodegradable polymer matrix such as collagen, hydrogels such as alginate, hydron (hyaluronic acid) and polyethylene glycol polymers (See page 29827, column 2, first full paragraph, in particular). Dinbergs *et al* teach TGFβ is useful for eliciting extracellular matrix formation without increasing cellular proliferation for up to five days when coupling to various polymer such as alginate hydrogel for a sustained release (See page 29825, Fig 3A, in particular).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to use TGFβ at a concentration of 15-30 ng/ml as taught by Dinbergs *et al* for a method of making a tissue engineering scaffold for inducing formation of extracellular matrix by cells bound to the scaffold without increasing cellular proliferation as taught by the WO 96/27657 publication and Dinbergs *et al.* From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention.

One having ordinary skill in the art would have been motivated to do this because the WO 96/27657 publication teaches that when growth factor is localized to desired target cell, significantly less growth factor is needed to exert a biologic response (See abstract, in particular). Dinbergs *et al* teach that TGFβ coupling to polymer at a concentration of 15-30 ng/ml is useful for eliciting extracellular matrix formation without increasing cellular proliferation or cell number (See page 29825, Figures3A (Endothelial cell) and 3B (smooth muscle cell), comparing filled circle to the open circle which is the control, in particular). Claim 9 is included in this rejection because between 4×10^{-6} to 4×10^{-3} nmol/ml is equivalent to between 5 and 100 ng/ml and Dinbergs *et al* teach TGFβ at 15-30 ng/ml is effective for inducing formation of extracellular matrix by endothelial cell and smooth muscle cell without increasing cell proliferation.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

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